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Preclinical Study of Nanostructured Cubic Liquid Crystalline Formulating of *Ulva Fascista* Bioactive Polysaccharides against Hepatocirrhosis

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Abstract

From our previous study, the aqueous soluble polysaccharides isolated from cold aqueous extract of Ulva fasciata was prepared and represented as the most of its bioactive constituents. The polysaccharides were formulated as cubosomal nanoparticles for use as anti-hepatocirrhosis drugs. The formulations were characterized by their encapsulation efficiency, particle size, zeta potential and in vitro release. The selected formulation was subjected to a preclinical study. Serum biomarkers enzymes (aspartate and alanine aminotransferases and alkaline phosphatase) were proved the efficacy of the polysaccharides loaded cubosome similar to the reference drug; silymarin in addition to its safety on liver. The histopathological examination was conducted to document the biochemical results.

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Introduction

Although, some herbal supplements, including silymarin (an extract of milk thistle), are commonly used in chronic liver disease patients [1,2], there are limited available empirical data about the beneficial effects of these supplements [3]. However, administration of EH0202, a mixture of four herbal extracts (Pumpkin seed extract, Safflower flower extract, Asian Plantain seed extract and Japanese Honeysuckle flower extract), is reported to induce interferon activity and reduce hepatitis-C-virus RNA levels in patients with high viral titers [4]. Furthermore, [5],

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reported that hepatoprotective effect of birch bark extract in patients with Safflower flower extract Japanese Honeysuckle flower extract was proved. Sulfated polysaccharides, either from plants or algae, have a wide range of important biological properties especially for hepatic diseases. Marine algae are a rich source of sulfated polysaccharides with novel structures. A number of sulphated galectins (red algae), sulphated furans (brown algae) and sulphated oxides (green algae) are included in the list of the most abundant non- mammalian sulphated polysaccharides found in nature. These polysaccharides exhibited antiviral, reduced hepatic fibrosis, antioxidant properties (hepatoprotective), hypolipidemic and antitumor activities [6,7]. Evaluated the effects of fucoidan (a complex sulfated polysaccharide extract from marine brown seaweed) on hepatitis C virus (HCV) RNA load both *in vitro* and clinically and found that fucoidan inhibited the expression of HCV replicon *in vitro* in a dose dependent manner. Furthermore, HCV RNA levels were significantly lower relative to the baseline and lower serum alanine aminotransferase levels after 8-10 month of treatment with fucoidan. There are over 100 million people with hepatic fibrosis in the world. Hepatic fibrosis results from chronic damage to the liver in conjunction with the progressive accumulation of fibrillar extracellular matrix proteins. Administration of fucoidan reduced CCl4-induced acute and chronic liver failure (c). In addition, polysaccharides of Gracillariid corticate protect the liver from aflatoxin B_1 (AFB_1) toxicity [8]. Damage to hepatocytes and activation of hepatic stellate cells are key events in liver fibrosis, and, interestingly, treatment of hepatocytes with fucoidan prevented CCl4-induced cell death and inhibited the proliferation of hepatic stellate cells. So fucoidan might be a promising anti-fibrotic agent possessing dual functions, namely, protection of hepatocytes and inhibition of hepatic stellate cell proliferation [9]. Fucoidan prevented concanavalin A-induced liver injury by mediating the endogenous interleukin (IL)-10 productions and the inhibition of proinflammatory cytokine in mice [10,11]. Our previous studies had proved the antioxidant and anti-fibrotic activities of certain polysaccharide isolated from *Ulva fasciata*, *Enteromorpha intestinalis* and Dictyopterans membranate [12,13]. Seaweed which spread on Egyptian shores, their bioactive products is a storehouse of healthy attributes. The sulphated polysaccharide is considered as the treasure source for novel therapeutic agents. It has exhibited miraculous biological properties such as antiviral, prevented or reduced hepatic fibrosis, antioxidant properties or hepatoprotective, hypolipidemic and antitumor activities. Our strategy aimed to develop drugs more safe, efficient and cheaper from local marine algae to stimulate liver function; protect liver from damage and regeneration of hepatic cells. Recently, cubosomes which is a lipid-based drug delivery system has attracted attention owing to their stability and biocompatibility [14]. They are nanostructured liquid crystalline particles made of certain amphiphilic lipid and stabilized by poloxamers [15]. Cubosomes are also found to be promising vehicles for various routes of administration with regard to their ability to encapsulate hydrophilic, hydrophobic and amphiphilic substances, and the potential for controlled release through functionalization. [15]. For this reason, this study will discuss an easy way to prepare a cubic phase gel matrix containing polysaccharide isolated from *Ulva Fascista* cold aqueous extract, accompanied with a preclinical study of the formulated bioactive algal polysaccharides isolated against hepatocirrhosis.

Materials and Methods

Materials

A polysaccharide isolated from the cold aqueous extract of *Ulva Fascista* as mentioned in [16]. Glyceryl mono-oleate, Poloxamer 407

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and Poloxamer 188 were purchased from Sigma-Aldrich Chemical Company. Dialysis tubing cellulose membrane (molecular weight cut-off 12,000-14,000 g/mole), was purchased from Sigma-Aldrich Chemical Company, St. Louis, USA. All other reagents were of analytical grade.

Assessment of extract calibration curve using the Sulfuric acid - UV method

The procedure of the sulfuric Acid-UV method was performed as previously displayed in our published work [17].

Preparation of cubosomes

In a water bath, GMO and 0.25 g of either poloxamer 407 or poloxamer 188 were melted at 70 °C. The obtained molten solution was added drop wise to 4 ml of deionized water (70 °C) containing 50 or 100 mg of extract and vortexed. The solution was mixed at high speed at room temperature to achieve a homogenous state. The mixture was equilibrated at room temperature for 48 h to obtain the cubic gel [18]. Then, the cubic gel was dispersed with 18.50 ml deionized water by vortex at high speed for 3 min. The final concentration of lipid in the dispersion is 10% (*w/w*) with respect to the final dispersion weight. Four formulations have been prepared; the composition of the investigated autosomal nanoparticles is shown in table 1.

Table1: Different formulations of autosomal nanoparticles.

Formulations		**Surfactant type**		**Drug Conc.(mg)**	
		Poloxamer 407(gm)	**Poloxamer 188(gm)**		
U. fasciata cold polysaccharide extract drug	**F1**	0.25	-	50	-
	F2	0.25	-	-	100
	F3	-	0.25	50	-
	F4	-	0.25	-	100

Characterization of cubosomes

Particle size analysis

The average diameter of cubosomes dispersions was determined by photon correlation spectroscopy (PCS) using a Zetasizer Nano ZS (Malvern Instruments, Malvern, UK) at a fixed angle of 90 º and at 25 ºC. The aqueous cubosomal dispersions were diluted with distilled water before analysis. Each value represented as the average of 3 measurements.

Determination of drug entrapment efficiency

The entrapment efficiency was calculated by measuring the concentration of drug in the supernatant after centrifugation using cooling centrifuge (Union 32R, Korea). The unentrapped drug concentration was calculated by adding 1 ml of drug loaded cubic gel to 9 ml water and then centrifuging this dispersion at 9000 rpm at 4 ºC for 30 minutes. The supernatant was collected, filtered through Millipore membrane filter (0.2 µm) then diluted with water and measured against blank using the Sulfuric Acid UV method. The entrapment efficiency was determined using the following equation [19-21,40].

$$E.E = \frac{Initial\ drug\ conc. - free\ drug\ conc.}{Initial\ drug\ conc.}\ x\ 100$$

In vitro drug release studies

The dialysis bag diffusion technique previously reported by [22] was used for the calculation of the amount of polysaccharide released from the different cubosomal dispersions. The dialysis

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bag (molecular weight cutoff 12,000-14,000) filled with the cubosomal dispersion equivalent to 2 mg and sealed at both ends. The bag was then immersed in the receptor compartment containing 50 ml of phosphate buffer pH 6.8, stirred at 100 rpm and maintained at 37±2 ºC. At fixed time intervals ((0.5,1,2,3,4,5, and 6 hr.), 2 ml of the dissolution medium were taken from the receptor compartment and replaced with the same volume of fresh dissolution medium. The polysaccharide in the samples was spectrophotometrically measured at 322 nm. For all formulations, the release studies were tripled and the results were expressed as the mean values ±SD.

Transmission electron microscopy (TEM)

The morphology of the selected cubosomal formulation was tested using the transmission electron microscope. On cupper grid, one drop of the diluted sample was placed for inspection and stained with 2 % (W/V) phosphotungstic acid.

Differential scanning calorimetry (DSC)

DSC was performed on the drug loaded cubic gel, blank cubic gel, polysaccharide and poloxamer 407 using a thermal analysis system (DSC-60, Shimadzu, Japan) in order to detect any change in the physical state of the drug loaded cubic gel. The samples (5 mg) were heated at a constant rate of 10 °C/min in an aluminum pan and heated from 25 to 400 °C under a nitrogen atmosphere using differential scanning calorimeter. The thermogram obtained was evaluated for peak shift or appearance/disappearance of new peak.

Preclinical study

Animals

For this study, male Wistar albino rats (100-120g) were selected from the animal House, National Research Centre, Egypt. All the animals were housed in a controlled environment of air and temperature with access to water and diet. The experiment was complied according to the ethical guidelines of Medical Ethical Committee of the National Research Centre.

Experimental design

48 male rats were used for this study and classified into 8 groups (6 rats in each group) as following:

- **Group1**: served as normal control rats.
- **Group2**: served as CCl_4 intoxicated rats. Rats intraperitoneally injected with 0.5 ml CCl_4/kg body weight (1:9 v/v olive oil) two times/ week for 6 consecutive weeks [23].
- **Groups 3 and 4** (recovery group): served as CCl_4 intoxicated rats for 6 weeks (as group 2) and left free for either 15 or 30 days, respectively.
- **Group 5**: served as CCl_4 intoxicated rats for 6 weeks, and then orally treated with Cubic liquid crystalline nanoparticle of polysaccharide isolated from cold aqueous extract of *Ulva Fascista* (50 mg/kg b. wt./ daily for 15 days).
- **Group 6**: served as CCl_4 intoxicated rats for 6 weeks, and then orally treated with Cubic liquid crystalline nanoparticle of polysaccharide isolated from cold aqueous extract of *Ulva Fasciata* (50 mg/kg b.wt./ daily for 30 days).
- **Groups 7 and 8**: served as CCl_4 intoxicated rats for 6 weeks, and then orally treated with silymarin drug (100 mg/kg b. wt) daily for 15 and 30 days, respectively [24].

Biochemical determinations

Aspartate and alanine aminotransferases (AST, ALT) and alkaline phosphatase (ALP) were estimated by the method of Gella et al. And Rusalki et al. [26], respectively.

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Histopathological examination of processed liver samples

In 10% buffered formalin, liver tissues were fixed and processed until embedded in paraffin. From the prepared paraffin blocks, serial liver sections with a thickness of 4 µm were obtained. The liver sections were stained with hematoxylin & eosin and Masson 'trichome stains. A Zeiss microscope was used to conduct the histopathological examination of the stained sections. [27].

Statistical analysis

Data analysis was carried out by unpaired t-test and one-way analysis of variance (ANOVA) followed by post-hoc analysis at least significant difference (LSD) between groups at $p < 0.05$ using Costate software (USA) Computer Program.

Results and Discussion

Characterization of cabooses

Particle size analysis

The results of particle size are presented in Table 2. All the prepared formulations are in the nano-size range.

Determination of drug entrapment efficiency

Table 2 elucidates the entrapment efficiencies of the prepared formulations. Many factors such as the type and concentration of surfactant as well as the concentration of the drug were studied to evaluate their effects on the formulations. Regarding the *U. fasciata* cold polysaccharide extract drug, presence of poloxamer 188 show a significant increase of entrapment efficiency compared to poloxamer 407 at constant amount of drug. Table 2 shows a significant increase in the entrapment efficiencies of drug cubosomes nanoparticles increase with increasing the drug from 50 mg to 100 mg.

Table 2: Entrapment efficiency, Particle size and Zeta potential of autosomal preparations.

Formulations	Entrapment efficiency (%±S.D.)	Particle size (nm ±S.D.)	Zeta potential (mV ±S.D.)
F1	42.75±3.63	275.4±10.22	-8.62
F2	54.4±7.35	212.4±9.33	-4.98
F3	46.20±2.54	328.9±15.01	-11.4
F4	64.04±.48	236.2±12.55	-3.69

In vitro release study

Figure 1 elucidates the *in vitro* release of polysaccharide from the loaded cubosomes nanoparticles. The moderately slow release of active moieties observed from cubosomes may be attributed to the limited diffusion of polysaccharide integrated in the aqueous channels; in which case diffusion is regulated by the aqueous channel tortuosity and the relatively narrow pore size. [28,29]. Previous studies reported the potential of cubosomes to provide a slow-release matrix for drugs of varying sizes and polarity [30,31,32]. Also, the presence of GMO contributes in the slow release of the drug from the cubosomes nanoparticles, which might lead to slower partitioning of the drug from the oily medium to the aqueous one [33]. As shown in figure 1, release of the *U. fasciata* cold polysaccharide extract moiety is dependent on both drug concentration and type of surfactant. Increasing drug concentration led to a concomitant increase in percentage drug released in cubosomes prepared with either surfactant. It was clear that surfactant type had a great impact on drug release; cabooses prepared using poloxamer 188 showed enhanced drug release property compared to poloxamer 407. This can be explained by the higher HLB value of poloxamer 188 compared to poloxamer 407 (>24 and 18-23, respectively). Increased HLB value of surfactant is expected it increase

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partitioning of the encapsulated drug from the oily medium to the aqueous one. Regarding these findings, F4 was selected for further characterization and investigations as it revealed the highest entrapment efficiency, small particle size and the highest percent of drug released.

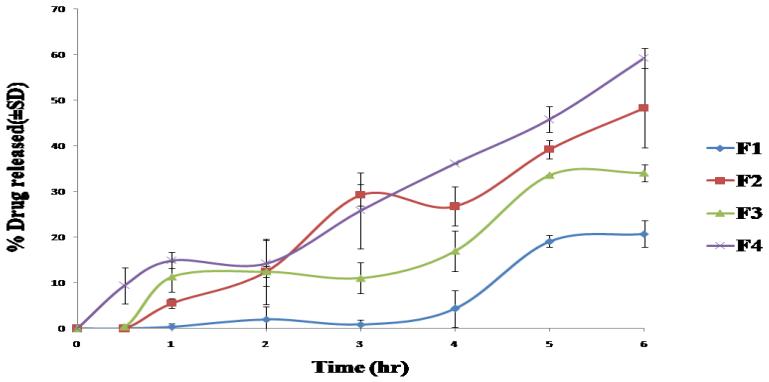

Figure 1: Drug release profiles from the prepared cubosomal formulations containing *Ulva fasciata* cold polysaccharide extract drug in phosphate buffer saline (pH 7.4) for 6 hr.

Transmission electron microscopy

The morphology of the cubosome was visualized using the transmission electron microscopy. As shown in figure 2, the characteristic caboose structure shows irregular hexagonal shape well dispersed.

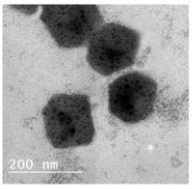

Figure 2: Transmission electron microscopy image of *Ulva fasciata* loaded cubosome.

Differential scanning calorimetry

The thermograms of *Ulva Fascista* extract, poloxamer 188, blank cubosomes and polysaccharide loaded cubosomes are shown in figure 3. It is evident that the polysaccharide and the poloxamer 188 show an endothermic melting point at 122.5 °C and 52°C, respectively. Meanwhile, F4 revealed complete disappearance for the peaks of the polysaccharide and the poloxamer indicating complete spreading of the polysaccharide in the cubosomes.

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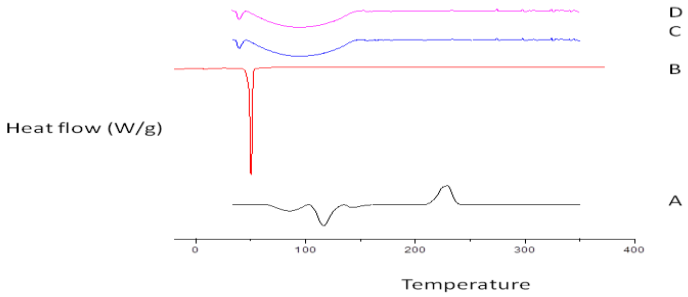

Figure 3: DSC thermograms of (A) *Ulva fasciata* extract, (B) Poloxamer 188, (C) Blank cubosome and (D) F4.

Preclinical study of Cubic liquid crystalline nanoparticle of polysaccharide isolated from cold aqueous extract of Ulva Fascista against hepatocirrhosis

From the sensitive indicators for liver injury are ALT and AST [34,35,36]**,** tables 3-5 revealed significant increase in liver function enzymes in CCl4 injured rats. After 45 days of intoxication with CCl4, AST, ALT and ALP recorded significant increase by 43.64, 115.69 and 42.54%, respectively as compared with the control rats. In the other intoxicated CCl4 groups, we noticed that AST, ALT and ALP decreased by 18.72, 8.93 and 2.74%, respectively when rats left free for 15 days after intoxication comparing with the 45 days CCl4 intoxicated group. In addition, AST, ALT and ALP showed decline in their activities by 29.72, 12.26 and 10.80%, respectively, when rats left free for 30 days after intoxication with CCl4 as compared with the 45 days CCl4 group. Therefore, we concluded that the liver undergoes more or less a recovery process with time. In agreement with our results, many studies reported a significant elevation of ALT and AST after CCl_4 intoxication [37,38]. Also, our finding recording significant increase in ALP after CCL4 intoxication. This was in agreement with Reyes-Gordillo *et al*. [39]. They returned that increase in serum enzymes to the increase in fluidity of the hepatic cell membrane that led to the release of enzymes into circulation. Treatments with polysaccharide loaded cubosomes (F4) and silymarin for 15 days improve AST, ALT and ALP by 54.97, 52.46 and 13.39%, respectively for F4, while silymarin recorded improvement by 67.95, 70.40 and 13.73%, respectively (Tables 3-5). While, treatments with F4 and silymarin for 30 days improve AST, ALT and ALP by 70.44, 69.05 and 23.27%, respectively for F4, while silymarin recorded improvement by 72.65, 73.99 and 23.12%, respectively (Tables 3-5). F4 showed similar hepatoprotective activity against fibrosis induced by CCl4 when compared with silymarin (Table 6). These results provide additional evidence for the fact that this formula is capable of conditioning hepatocytes, accelerating the regeneration of parenchymal cells, shielding them from membrane fragility, and reducing enzyme leakage into circulation. This extract therefore, behaved in the same mode of action as silymarin [38]; the standard herbal reference drug. Interestingly, cold polysaccharide extracts of *Ulva fasciata* recorded more potent effect in improving AST, ALT, and ALP (95.30, 68.87, and 115.06 %,

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respectively). Furthermore, our results revealed that treatments with F4 for 15 and 30 days give improvement of AST, ALT, and ALP similar to those of silymarin.

Table 3: Effect of treatment with F4 and silymarin drug on aspartate aminotransferase (AST) enzyme activity.

Groups	Mean ±SD	% change		% of improvement
		control	45 days CCl4	
Control	3.62^{de}±0.17	---	---	---
CCl4 (45 days)	5.82^{a}±0.16	+43.64	---	---
CCl4 (45 days+15 days free)	4.73^{b}±0.14	+30.66	-18.72	---
CCl4 (45 days+30 days free)	4.09^{c}±0.36	+21.98	-29.72	---
Treated (F4) for 15 days	3.83^{cd}±0.13	+5.80	-34.19	54.97
Treated (F4) for 30 days	3.72^{f}±0.14	-9.66	-43.81	70.44
Silymarin treated for 15 days	3.36^{ef}±0.10	-7.18	-42.26	67.95
Silymarin treated for 30 days	3.19^{f}±0.05	-11.87	-45.18	72.65

Data are means ± SD of six rats in each group.
Statistical analysis is carried out by one way analysis of variance (ANOVA) accompanied by LSD significant difference at $p < 0.05$ using Costate Computer Program.
Unshared superscript letters are significant values between groups at $p < 0.0001$.

Table 4: Effect of treatment with F4 and silymarin drug on alanine aminotransferase (ALT) enzyme activity.

Groups	Mean ±SD	% change		% of improvement
		control	45 days CCl4	
Control	2.23^{f}±0.07	---	---	---
CCl4 (45 days)	4.81^{a}±0.09	+115.69	---	---
CCl4 (45 days+15 days free)	4.38^{b}±0.09	+96.41	-8.93	---
CCl4 (45 days+30 days free)	4.38^{c}±0.15	+89.23	-12.26	---
Treated (F4) for 15 days	4.22^{d}±0.09	+63.22	-24.32	52.46
Treated (F4) for 30 days	3.27^{e}±0.07	+46.63	-34.14	69.05
Silymarin treated for 15 days	3.24^{e}±0.02	+45.29	-32.46	70.40
Silymarin treated for 30 days	3.16^{e}±0.05	+41.70	-34.30	73.99

Data are means ± SD of six rats in each group.
Statistical analysis is carried out by one-way analysis of variance (ANOVA) accompanied by LSD significant difference at $p < 0.05$ using Costate Computer Program.
Unshared superscript letters are significant values between groups at $p < 0.0001$.

Histopathological examination

Figures 4 and 5 show the liver sections stained with hematoxylin &eosin (H&E) and Masson's trichome. Sinusoidal hepatocyte cards with central vein and portal tracts are seen in the normal liver tissue section. Portal triad with portal vein, hepatic artery and bile duct (A, A*&B, B*) are seen in the portal tracts. Distortion in the organization of cells around the central vein are observed in CCL4 group with periportal fatty infiltration and focal necrosis of hepatocytes (C, C*). On the other hand, cubosomal treatment brought back the cellular arrangement around the central vein and reduced necrosis. It also helped to bring the blood vessels to normal status (D, D*). Silymarin therapy reports hepatocyte

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regeneration with prominent nucleus and no signs of necrosis (E,E*). We have reported that histopathological evaluations confirmed biochemical results liver function enzymes in CCl4 injured rats showing abnormalities hepatocytes hypertrophied cell bodies, irregularities in the boundaries of central veins, a lot of apoptotic cell nuclei, many large vacuoles in the cytoplasm (Ibrahim et al., 2018) In addition, showing reduction in hepatic tissue injuries resulting from CCL4 when treated with polysaccharide loaded cubosomes or silymarin. This improvement was confirmed by our previous study on the polysaccharides isolated from *Spirulina platensis* alga [13].

Table 5: Effect of treatment with F4 and silymarin drug on alkaline phosphatase (ALP) enzyme activity.

Groups	Mean ±SD	% change		% of improvement
		control	45 days CCl4	
Control	170.41^{d}±9.81	---	---	---
CCl4 (45 days)	242.91^{a}±1.55	+42.54	---	---
CCl4 (45 days+15 days free)	236.24^{a}±2.03	+38.63	-2.74	---
CCl4 (45 days+30 days free)	216.66^{b}±2.56	+27.14	-10.80	---
Treated (F4) for 15 days	220.08^{b}±17.71	+29.14	-9.39	13.39
Treated (F4) for 30 days	203.25^{c}±5.37	+19.27	-16.32	23.27
Silymarin treated for 15 days	219.50^{b}±3.69	+28.80	-9.63	13.73
Silymarin treated for 30 days	203.50^{c}±5.80	+19.41	-16.22	23.12

Data are means ± SD of six rats in each group.
Statistical analysis is carried out by one-way analysis of variance (ANOVA) accompanied by LSD significant difference at p< o.05 using Costate Computer Program.
Unshared superscript letters are significant values between groups at p<0.0001.

Table 6. Relative Antifibrotic biomarkers Activity of (F4) to silymarin drug.

Groups	% of relative activity		
	AST	ALT	ALP
Treated (F4) for 15 days/ Treated silymarin for 15 days	80.90	74.92	97.50
Treated (F4) for 30 days/ Treated silymarin for 30 days	96.96	99.53	100.61

The activity of (F4) was evaluated according to the enzyme inhibition related to activity of silymarin. Activity>75% high, 75-50%: good, 50-25%: normal, <25%: weak activity.

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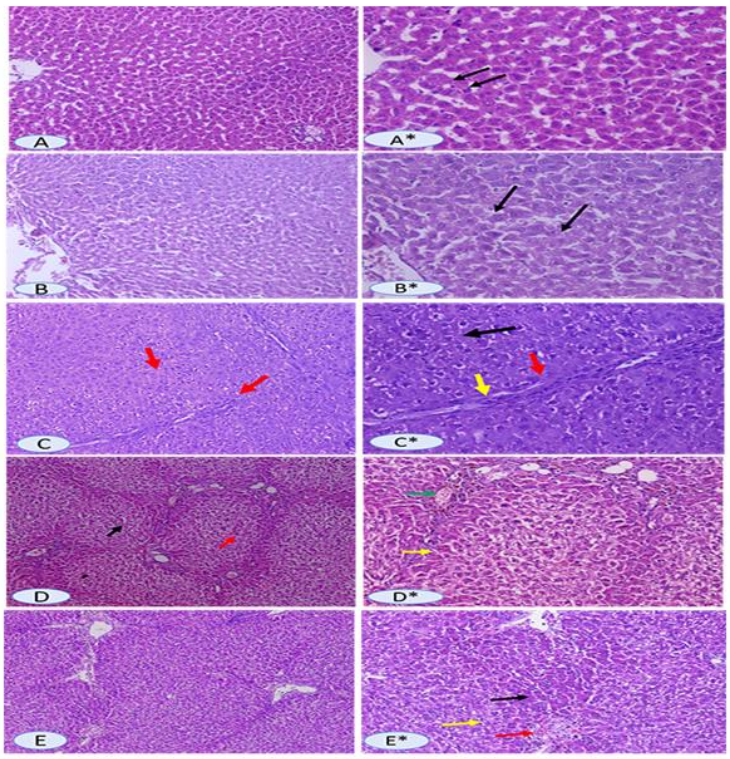

Figure 4: Liver sections stained with hematoxylin & eosin (H&E).

Normal control rat liver **(A, A*)** showed hepatic tissue with normal structure and architecture, (x200& x400). Control rat **(B, B*)** treated with polysaccharide loaded cubosomes showed hepatic tissue with normal structure and architecture (x200 & x400). CCl4 group **(C, C*)** showed distorted lobular hepatic architecture (black arrow) with attempt of nodule formation as thick interlobular septa and fibrosis (red arrow), with moderate ballooning of hepatocyte with (yellow arrow), and binucleated nuclei (black arrow), (x200 & x400). CCl4 group (**D, D***) treated with polysaccharide loaded cubosomes showed more or less normal hepatic lobular architecture, formation of small (black arrow) and large (red arrow) complete and incomplete hepatic nodules and mild fibrous tissue (yellow arrow) in portal tract (green arrow) (x100 & x200). Silymarin group **(E, E*)** showed preserved (intact) lobular hepatic architecture with thin plates of normal hepatocytes (black arrow) and mild ballooning of hepatocytes (yellow arrow), portal tract (red arrow) (x100 & x200).

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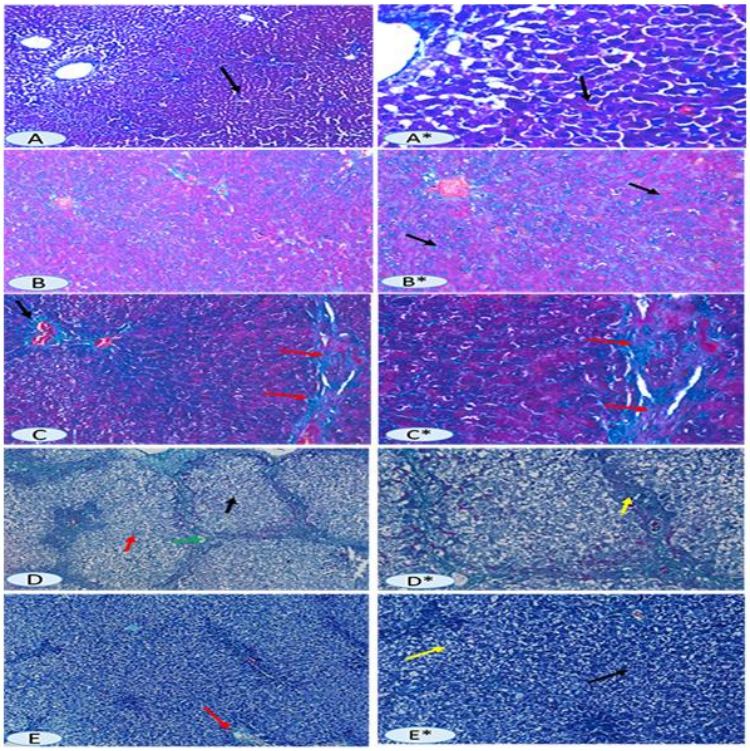

Figure 5: Liver sections stained with Masson's trichome.

Normal control rat liver **(A, A*)** showed hepatic tissue with normal structure and architecture, hepatocytes arranged in thin plates (black arrow) (x100 & x400). Control rats **(B, B*)** treated with polysaccharide loaded cubosomes showed hepatic tissue with normal structure and architecture, hepatocytes arranged in thin plates (black arrow) (x200& x400). CCl4 group **(C, C*)** showed distorted lobular hepatic architecture (black arrow) with attempt of nodule formation as thick interlobular septa and fibrosis (red arrow), with thick portal tract (black arrow) (x200 & x400). CCl4 group **(D, D*)** treated with polysaccharide loaded cubosomes showed intact hepatocytes, formation of small (black arrow) and large (red arrow) complete and incomplete hepatic nodules, mild fibrous tissue (yellow arrow) in portal tract (green arrow) (x100 & x200). Liver section from silymarin group **(I, J)** showed preserved (intact) lobular hepatic architecture with thin plates of normal hepatocytes (black arrow) and mild ballooning of hepatocytes (yellow arrow), portal tract (red arrow) (x100 & x200).

Conclusion

Cold extract of polysaccharide *Ulva Fascista* was successfully loaded with cubic liquid crystalline nanoparticles. The selected formulation showing high entrapment efficiency, small particle size and high percent of polysaccharide released was subjected to a preclinical study against intoxicated rate with CCL4 compared to Silymarin as a reference drug. Our findings indicate that treatment with

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Ulva fasciata (cold extract) attenuated the elevated serum enzymes and resulted in a subsequent recovery towards normalization.

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